Relationships Between Volatile Compounds, Storage Conditions, Deodorization, and Flavor Scores of Milk Fats and Dry Whole Milks[1]

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Abstract

Butterfat, deodorized butterfat, dry whole milk, and a dry whole milk-like product containing 26% deodorized butterfat were stored for 3 and 6 mo at 4 and 27 C. Initially and after each storage period, samples were evaluated for flavor after reconstituting to whole milk with 3.3% fat, the butterfats by homogenizing with skim milk, and the powders by reconstituting with water. At the same periods the butterfats and fats extracted from powders were steam deodorized at 50 C and 1 torr, and the volatile compounds were collected and gas chromatographed. The chief volatile compounds, quantitatively, were identified as caproic, caprylic, capric, and lauric acids, nonanone, undecanone, tridecanone, and pentadecanone methyl ketones, and octa, deca, dodeca, and tetradeca delta lactones. Their concentrations are reported, and relationships to flavor scores were examined. Lactone concentration, deodorization, storage time and temperature and deodorization-temperature interaction were significant factors. Flavor scores after storage were related, in a linear model, to numerical values for these significant factors.

Introduction

Dry whole milk (DWM) can be protected from ozone during manufacture (3) and from oxygen during storage (10). Nonoxidative off-flavors formed during storage remain its major flavor problem. The fat phase appears to be the chief source of these off-flavors which, to a great extent, are caused by volatile compounds formed from nonvolatile precursors (8, 9).

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Volatile amounts and intensity of off-flavor in butterfat are related (9). Our work has attempted more specific correlation and has been expanded to include butterfat, deodorized butterfat, DWM, and deodorized DWM, a product analogous to DWM prepared by homogenizing a suitable mixture of deodorized butterfat and skim milk, concentrating, and drying.

Materials and Methods

Butterfat, deodorized butterfat, DWM, and deodorized DWM were prepared as previously described: the butterfat by the continuous-line, phase-inverter method and the deodorized DWM from deodorized fat which was protected at all times from exposure to air (4). Fats were stored in nitrogen and powders in an oxygen-scavenging system (4). Acids, ketones, and lactones were used as purchased (2). Grades and treatments of solvents and reagents

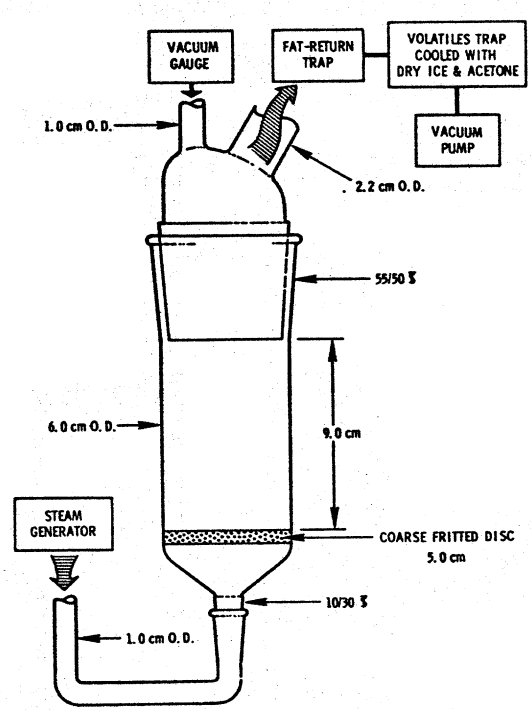

FIG. 1. Apparatus for deodorizing small fat samples.

TABLE 1. Recovery of fatty acids from butterfats and dry whole milks.[a]

Fatty acid	Butterfat[b] Initial	3 mo storage 4 C	3 mo storage 27 C	6 mo storage 4 C	6 mo storage 27 C	Deodorized butterfat[b] Initial	3 mo storage 4 C	3 mo storage 27 C	6 mo storage 4 C	6 mo storage 27 C
C_4	1.7	.0	.0	4.9	.0	.0	.0	.0	.0	.0
C_6	.9	1.2	2.1	1.6	2.4	.0	.0	.1	.3	.4
C_{10}	9.0	8.4	8.4	11.9	13.9	.1	.3	.3	.1	.4
C_{12}	4.4	3.8	4.0	5.5	5.6	.4	.0	.0	.0	.0
	Dry whole milk[c]					Deodorized dry whole milk[c]				
C_4	88.5	88.5	127	158	333	62.3	138	146	150	277
C_6	20.8	18.5	26.9	36.5	41.9	21.2	24.6	28.1	33.8	57.7
C_{10}	25.0	22.7	45.4	41.2	65.8	20.4	21.5	28.8	4.2	59.2
C_{12}	9.2	8.5	14.2	13.8	20.0	8.1	4.6	6.9	5.8	15.0

[a] Averages of duplicate experiments.
[b] Expressed as mg acid/kg butterfat.
[c] Expressed as mg acid/kg extracted fat.

TABLE 2. Recovery of ketones from butterfats and dry whole milks.[a]

Ketone	Butterfat[b] Initial	3 mo storage 4 C	3 mo storage 27 C	6 mo storage 4 C	6 mo storage 27 C	Deodorized butterfat[b] Initial	3 mo storage 4 C	3 mo storage 27 C	6 mo storage 4 C	6 mo storage 27 C
C_9	.20	.10	.15	.15	.25	.12	.05	.12	.10	.12
C_{11}	.30	.25	.40	.35	.65	.17	.17	.25	.15	.25
C_{13}	.85	.70	.75	1.30	1.40	.35	.30	.30	.40	.50
C_{15}	.95	.90	1.25	1.30	1.75	.75	.70	.65	.55	.80
	Dry whole milk[c]					Deodorized dry whole milk[c]				
C_9	.58	.46	.00	.15	.19	.58	.58	.58	.15	.15
C_{11}	.58	.38	.58	.00	.00	.58	.19	.38	.00	.00
C_{13}	.58	.58	.96	.00	.00	1.15	.96	1.15	.00	1.35
C_{15}	.38	.77	1.73	.58	2.31	1.92	1.15	1.73	.96	2.88

[a] Averages of duplicate experiments.
[b] Expressed as mg ketone/kg butterfat.
[c] Expressed as mg ketone/kg extracted fat.

TABLE 3. Recovery of delta lactones from butterfats and dry whole milks.[a]

Lactone	Butterfat[b] Initial	3 mo storage 4 C	3 mo storage 27 C	6 mo storage 4 C	6 mo storage 27 C	Deodorized butterfat[b] Initial	3 mo storage 4 C	3 mo storage 27 C	6 mo storage 4 C	6 mo storage 27 C
C_8	.5	.5	.8	.7	1.1	.0	.0	.0	.1	.0
C_{10}	5.3	5.8	8.1	8.6	11.5	.3	.5	.9	.4	1.1
C_{12}	5.3	4.6	7.6	7.0	9.6	.6	.7	1.1	.3	1.3
C_{14}	1.4	1.1	1.6	1.3	1.8	.4	.3	.4	.3	.6
	Dry whole milk[c]					Deodorized dry whole milk[c]				
C_8	.8	.4	1.5	1.9	2.3	.0	.4	.4	1.6	1.6
C_{10}	2.3	5.0	8.8	8.5	10.4	1.6	1.2	1.6	1.9	2.7
C_{12}	2.3	4.6	9.2	6.2	8.8	1.9	1.2	2.3	2.7	3.1
C_{14}	.8	1.2	1.9	1.5	1.2	1.2	.8	1.2	1.2	1.6

[a] Averages of duplicate experiments.
[b] Expressed as mg lactone/kg butterfat.
[c] Expressed as mg lactone/kg extracted fat.

have been described (2, 9).

Fat samples were extracted from powders with methylene chloride after a preliminary hydration of lactose (1). Each fat sample (25 g) was steam deodorized at 50 C and 1 torr for 3 h with a steam flow of 50 g/h. The aqueous distillate was extracted with methylene chloride, the extract concentrated, and an aliquot subjected to gas chromatography on an Apiezon L column (244 $\times$ .32 cm) with an 80 to 220 C program. Published details (9) have been modified only with respect to the apparatus (Fig. 1), weight of fat, and amount of steam.

Identities were assigned to the 12 major peaks by comparing their retention times with those of known compounds. Uncertainties, from close association of peaks, were resolved by separating the volatiles into chemical classes (2) and rechromatographing. Each peak was quantitated by comparing its height with a dilution-series curve of the corresponding compound (2). Individual results of duplicate experiments varied considerably because of the different samples and the many techniques. However, the same trends leading to the conclusions appeared in both experiments. Averages of duplicate values, reported in Tables 1 to 3 still show some unexpected variation remaining from the additive errors.

Analysis of variance and mathematical correlations were obtained with computer techniques described by Mendenhall (6).

Samples were evaluated initially and after storage as indicated in the tables. All organoleptic evaluations were on whole milks (3.3% fat) prepared by reconstituting the powders with water and homogenizing the fats with skim milk (8) in a Manton-Gaulin Model 15-M homogenizer.[3] Samples were held at 4 C for about 20 h, then warmed quickly to room temperature. and tasted by a 10-member panel of experienced judges using a scoring range of 31 to 40 (5). All samples were tasted blind. with no more than 10 samples, including duplicates, presented at one time.

Results and Discussion

In our gas chromatograms, adjacent members of each chemical class had widely different retention times. However, occasional difficulties in identification were encountered when two compounds in different chemical classes had similar retention times. These uncertainties were resolved by separating the combined volatiles into chemical classes (2), separately chromatographing each chemical class, and comparing the retention times and peak heights in the individual fractions with those in the combined volatiles.

The major chromatographic peaks were identified as representing the normal saturated C_6, C_8, C_{10}, and C_{12} fatty acids; C_9, C_{11}, C_{13}, and C_{15} methyl ketones; and C_8, C_{10}, C_{12}, and C_{14} Δ-lactones. These compounds recovered from butterfats and milk powders held under various storage conditions are in Tables 1 to 3. Recoveries from butterfats are expressed as mg compound/kg butterfat and from the powders as mg compound/kg extracted fat. Compounds with minor peaks such as gamma-lactones were not included because of quantitative insignificance. Low boiling compounds could not be measured if their peaks were obscured by the large solvent peak at the start of the chromatograms.

We stress that steam deodorizations were under two sets of conditions. To minimize the formation of artifacts, fat samples deodorized for analysis were deodorized at 50 C and 1 torr for 3 h. In contrast, fats deodorized to degrade lactone and ketone precursors were deodorized at 150 C and 2 torr for 6 h. Under the mild conditions (50 C), we would expect recovery of higher boiling compounds to be incomplete.

In our procedure for extracting fat from DWM, fatty acids in the fat phase are extracted as completely as the fat – those in the nonfat phase, being at least partially equilibrated with their salts, are only partially extracted. Finally, C_4 acid is completely lost and C_6 acid partially lost from methylene chloride extracts of steam distillates (2).

Generally, fatty acids increased only slightly in butterfat during storage and hardly at all in deodorized butterfat. This latter result may be due to low moisture in deodorized fat because of the drying effect of superheated steam (150 C at 1 torr).

Fatty acids from DWM, initially and after storage (Tables 1 and 4), were much higher than those from butterfat. This indicates the non-fat phase must be the major source of these acids in DWM. Since fatty acids generated during storage must all arise in the fat, their greater generation in DWM implies that the nonfat phase accelerates the decomposition of fatty acid triglycerides. To show this more clearly, part of the data of Table 1 has been recalculated (Table 4). The higher moisture in DWM compared to that in butterfat is one factor contributing to accelerated decomposi-

[3] Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

TABLE 4. Recovery of fatty acids generated in butterfats and dry whole milks during storage at 27 C for 6 mo.

	Product							
	Butterfat		Deodorized butterfat		Dry whole milk		Deodorized dry whole milk	
Fatty acid	mg acid/ kg fat[a]	mg acid/kg acid potential[b]	mg acid/ kg fat[a]	mg acid/kg acid potential[b]	mg acid/ kg fat[a]	mg acid/kg acid potential[b]	mg acid/ kg fat[a]	mg acid/kg acid potential[b]
C_6	...[c]	...[c]	.0	.0	245	11,021	215	9,672
C_8	1.5	149	.4	39.7	21.1	2,095	36.5	3,625
C_{10}	4.9	170	.3	10.4	40.8	1,413	38.8	1,343
C_{12}	1.2	44.0	...[c]	...[c]	10.8	396	6.9	253

[a] Calculated from data of Table 1 as difference between initial values and those after storage.

[b] Calculated on assumption that fat sample contained a total fatty acid potential equal to 95% of its weight and that of this potential C_6 acid equaled 2.34%, C_8 acid 1.06%, C_{10} acid 3.04%, and C_{12} acid 2.87%.

[c] Negative values.

tion of fatty acid triglycerides in DWM. Probably of greater importance is the ability of the nonfat phase to act as a reservoir for newly formed fatty acids by converting a significant portion of them into salts, thus continuously disturbing the equilibrium in the fat between glycerol hydroxyl groups, fatty acids, and water. This view is supported by data in Table 4 which show a clear correlation between molecular weight, hence, diffusibility of fatty acids and their rate of generation.

Correlations. Flavor scores were correlated with measured volatile compounds and with experimentally independent variables of deodorization, storage temperature, storage time, and type of product (butterfat or DWM). As a preliminary, to simplify the statistical analysis, volatile compounds were compared with published flavor thresholds (Table 5). The flavor thresholds were determined with aqueous

TABLE 5. Volatiles in reconstituted milks and their flavor thresholds.

	Source of milk				
	Fat + skim milk		DWM[a] + water		
	Volatiles[b]				Flavor
Volatile	Initial	Highest[c]	Initial	Highest[c]	Threshold[d]
C_6 acid	.06	.16	2.88	10.9	15
C_8 acid	.03	.80	.68	1.4	5.8
C_{10} acid	.30	.46	.81	2.1	3.5
C_{12} acid	.15	.19	.30	.65	...
C_9 ketone	.007	.007	.025	.025	.19
C_{11} ketone	.010	.04	.025	.025	.45
C_{13} ketone	.030	.17	.025	.038	.50
C_{15} ketone	.033	.09	.013	.075	...
C_8 lactone	.017	.037	.025	.075	.57
C_{10} lactone	.18	.38	.075	.34	.16
C_{12} lactone	.18	.32	.075	.30	.10
C_{14} lactone	.047	.06	.025	.063	9.8

[a] DWM = dry whole milk.

[b] As mg/liter in milk containing 3.3% fat. In milk reconstituted from fat + skim milk, the contribution of the skim milk has been disregarded.

[c] Usually but not always in samples stored 6 mo at 27 C.

[d] As mg/liter aqueous solution giving 50% response. Data from Siek et al. (7).

TABLE 6. Analysis of variance of flavor scores.

Source	Degrees of freedom	Sum of squares
L** (lactone)	1	11.128
D** (deodorization)	1	2.556
T** (storage temperature)	1	1.369
DT** (deodorization-temperature interaction)	1	.976
t* (storage time)	1	.673
FT (product-temperature interaction)	1	.290
DF (deodorization-product interaction	1	.177
Tt (storage temperature-time interaction)	1	.037
Dt (deodorization-storage time interaction)	1	.033
Ft (product-storage time interaction)	1	.008
F[a] (product)	1	.000
Error	19	1.987
Total	30	19.234

** Significant at 1%.

* Significant at 5%.

[a] F, alone or in interaction terms, refers to butterfat or dry whole milk as the product used for flavor evaluation or as the source of fat from which volatiles were collected.

Table 7. Terms of best model, their description and numerical values.

Term	Description	Numerical values			
γ	calculated score	calculated			
μ	mean score	35.758			
D	deodorization	deodorized .396	not deodorized (−) .396		
T	storage temperature	4 C .219	27 C (−) .219		
t	storage time	3 mo .163	6 mo (−) .163		
β	lactone coefficient	(−) .0418			
L	lactone[a]	measured			
DT	deodorization-temperature interaction	deodorized (4 C) (−) .179	undeodorized (4 C) .179	deodorized (27 C) .179	undeodorized (27 C) (−) .179
	random effect				

[a] Milligrams combined lactones/kg product. For each set of conditions, equal to sum of four lactones recovered from butterfats (Table 3) and to 26% of sum of four lactones recovered from extracted fats of dried milks (Table 3).

solutions; volatile compounds were equilibrated in milk to an unknown extent between fat and a buffered aqueous solution. With these limitations, the comparison only can suggest possibilities. The indications are that the ketones did not contribute to off-flavors, the fatty acids might have contributed, and the lactones probably did contribute to off-flavors.

In one experiment, we separated the volatiles from stale butterfat into two chemical classes (2), one containing the lactones, the other containing both ketones and fatty acids. When added to fresh milk, only the lactone fraction produced a detectable off-flavor.

We elected to include two classes of volatiles: total lactones (combined four lactones) and total volatiles (combined 12 compounds). Each class of volatiles was mg/kg butterfat or mg/kg DWM.

The analysis of variance is summarized in Table 6. Differences in flavor scores with lactones, deodorization, storage temperature, and deodorization-temperature interaction were significant at 1% and storage time at 5%. The multiple correlation equaled .87.

To correlate flavor scores with variables of our study, more than 70 linear models were tested. Total volatiles were nonsignificant and,

Table 8. Actual and calculated flavor scores of variously treated milkfats and dry whole milks (DWM).[a]

Product	Storage temperature (C)	Initial actual	Flavor score 3 mo calculated	3 mo actual	6 mo calculated	6 mo actual
Undeodorized fat	4	36.1	35.4	35.4	34.9	34.9
Undeodorized fat	27		34.4	34.3	33.8	34.0
Deodorized fat	4	36.3	36.3	36.4	36.0	35.9
Deodorized fat	27		36.4	36.8	35.8	35.7
DWM	4	37.1	35.8	35.9	35.4	35.4
DWM	27		34.9	34.9	34.5	34.5
Dried product with deodorized fat	4	37.1	36.3	36.6	35.9	36.5
Dried product with deodorized fat	27		36.2	36.2	35.9	35.9

[a] Data are averages of two experiments.

accordingly, are not included in the following best model. Descriptions and numerical values of the terms of this model:

$$Y_{ijklm} = \mu + D_i + T_k + t_l + (DT)_{ik} + \beta L_{ijkl} + \varepsilon_{ijklm}$$

are given in Table 7. The subscripts identify the variables coming into play in each term. They are: i (deodorization), j (product), k (storage temperature), l (storage time), and L (lactone). From this model, calculated flavor scores and actual flavor scores are listed in Table 8.

Analysis of volatiles, because of its objectivity, may appear an attractive alternative to taste panel evaluations of stored butterfats and dry whole milks. We do not recommend it for this purpose because it is much more difficult (if skilled judges are available) and would require new models if preparations or storage conditions introduced off flavors not in our products.

Our dry milks have been developed for consumption as beverages and are judged in comparison with fresh milk. We believe the chief contribution of the relationships in our paper is to pinpoint and evaluate numerically the relative importance of the factors responsible for loss of fresh milk flavor during storage of this type of dry milk.

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